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ORIGINAL ARTICLE

Age-induced loss of wound-healing ability in potato tubers is partly regulated by ABA

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Abstract Wounding of potato (*Solanum tuberosum* L.) tubers induces the development of a suberized closing layer and wound periderm that resists desiccation and microbial invasion. Wound-healing ability declines with tuber age (storage period). The mechanism of loss in healing capacity with age is not known; however, upregulation of superoxide production, increased ABA biosynthesis and phenylalanine ammonia lyase (PAL) activity in response to wounding are processes critical to the development of a suberized closing layer and wound periderm. Therefore, the role of ABA in modulating the age-induced loss of wound-healing ability of tubers was examined. Non-wounded older tubers had 86% less ABA (dry matter basis) than younger tubers. PAL transcript increased in younger tubers within 24 h of wounding, but transcription was delayed by 5 days in older tubers. Wound-induced PAL activity increased more rapidly in younger than older tubers. ABA treatment increased PAL expression and activity in tissue from both ages of tubers and restored the 24 h transcription time line in older tubers. Moreover, ABA treatment of wounded older tubers enhanced their resistance to water vapor loss following a 6-day wound-healing period. Wound-induced accumulation of suberin poly(phenolic(s)) (SPP) and suberin poly(aliphatic(s)) (SPA) was measurably slower in older versus younger tubers. ABA treatment hastened SPP accumulation in older tubers to match that in younger tubers, but only enhanced SPA accumulations over the initial 4 days of healing. Age-induced loss of wound-healing ability is thus partly due to reduced ability to accumulate ABA and modulate the production of SPP through PAL in response to wounding and to dysfunction in the downstream signaling events that couple SPA biosynthesis and/or deposition to ABA. ABA treatment partly restored the healing ability of older tubers by enhancing the accumulation of SPP without restoring wound-induced superoxide forming ability to the level of younger tubers. The coupling of phenolic monomers into the poly(phenolic) domain of suberin was therefore not limited by the diminished wound-induced superoxide production of older tubers.

Keywords *Solanum tuberosum* · Wound healing · Suberization · Abscisic acid · Tuber age · Storage · Phenylalanine ammonia lyase

Abbreviations

ABA	Abscisic acid
Cys	Cysteine
NCED	9-*Cis*-epoxycarotenoid dioxygenase
NOX	NADPH oxidase
PAL	Phenylalanine ammonia lyase
SPA	Suberin poly(aliphatic(s))
SPP	Suberin poly(phenolic(s))

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Introduction

The wound-healing process in potato tubers is characterized by the development of a suberized closing layer and associated wound periderm. Suberin is a hydrophobic biopolymer rich in poly(phenolic) and poly(aliphatic) materials. The suberized cells and related hydrophobic matrices guard the underlying tissues from fungal and bacterial pathogens and serve as an efficient barrier against moisture loss (Vogt et al. 1983; Lulai and Orr 1994, 1995; Lulai and Corsini 1998; Matsuda et al. 2003; Schreiber et al. 2005). The ability of tubers to rapidly wound heal and develop an effective suberin barrier putatively declines with tuber age (storage at 4°C, 95% RH). Thus, comparative evaluation of wound-healing ability of tubers stored (aged) for varying periods can generate valuable insights into the physiological, biochemical and molecular processes that regulate the early responses to wounding and wound healing.

Phenylalanine ammonia lyase (PAL), an enzyme that catalyzes the first step in the phenylpropanoid pathway (Diallinas and Kanellis 1994; Kato et al. 2000), plays a crucial role in the wound-healing process of potato tubers (Hyodo 1976; Borchert 1978; Croteau et al. 2000; Kumar and Knowles 2003; Kumar et al. 2007b; Lulai et al. 2008). Tubers up-regulate PAL transcription and activity in response to wounding, which produces *trans*-cinnamic acid and *p*-coumaric acid monomers that are ultimately incorporated into the poly(phenolic)/aromatic (SPP) domain of suberin (Bernards 2002; Lulai 2007). It has been postulated that wound-induced superoxide radicals (Doke 1983; Miura et al. 1995; Johnson et al. 2003; Kumar and Knowles 2003) may participate in the peroxidase-mediated oxidative coupling of phenolic monomers into the aromatic domain of suberin (Lewis and Yamamoto 1990; Bernards et al. 1999; Razem and Bernards 2003; Kumar and Knowles 2003). This oxidative coupling mechanism per se remains equivocal, however, since superoxide production at the wound site is not sustained throughout the wound-healing period (Kumar et al. 2007b). After sufficient SPP material has accumulated on cell walls, suberin poly(aliphatics) (SPA) are laminated over the SPP domain (Lulai and Morgan 1992; Lulai 2007).

The ability to up-regulate superoxide production and PAL activity in response to wounding declines with tuber age (Kumar and Knowles 2003; Kumar et al. 2007b). Attenuation of wound-induced superoxide production compromises the ability of tubers to deter microbial infection (Kumar et al. 2007b). PAL activity is induced by various stresses such as pathogenesis, insect herbivory, wounding and exposure to low temperature (Dixon and Paiva 1995). Exogenous ABA induces PAL activity in other species (Jiang and Joyce 2003) and stimulates wound healing of potato tubers (Soliday et al. 1978). Endogenous ABA increases during wound healing (Soliday et al. 1978; Lulai et al. 2008) and fluridone-mediated inhibition of wound-induced ABA biosynthesis decreases PAL activity, effectively reducing the accumulation of suberin poly(phenolics) (Lulai et al. 2008). Importantly, addition of ABA to fluridone-treated tissue restored PAL activity and suberization (Lulai et al. 2008). ABA may therefore have a role in regulating the loss of wound-healing capacity with tuber age. We demonstrate that older tubers are indeed deficient in ABA and respond to exogenous ABA by up-regulating PAL and restoring SPP accumulation for suberin biosynthesis during wound healing. ABA treatment greatly improved the wound-healing ability of older tubers. However, ABA did not restore the age-induced reduction in wound-induced superoxide forming ability or SPA deposition.

Materials and methods

Plant material and preparation of tuber tissue for wound healing

Virus-free potato seed tubers (cv. Russet Burbank, certified Elite III from nuclear stock) were obtained directly from a seed grower at harvest (early October). The tubers were cured at 10°C (95% RH) for 10 days and then held at 4°C (95% RH) for a total storage period of 6–18 months.

The effects of tuber age and exogenous ABA on wound-healing ability were determined over a 6-day healing period. Tubers (150–200 g) were blocked for size into three replicates (3 tubers per replicate), cut into thirds perpendicular to the apical to basal axis, and the apical and basal portions discarded. Slices, 3-mm thick, were cut from the central third of each tuber with an electric food slicer. The tuber slices were stacked and then cored ca. 2 mm inwards from the periderm with a cork borer to produce 1.7-cm diameter discs. The discs were rinsed free of surface starch and immersed in milli-Q (Millipore Corp., Bedford, MA, USA) water with or without 100 μM (±)ABA for 5 min. This concentration of ABA was previously determined to be optimal for regulation of suberization (Soliday et al. 1978; Cottle and Kollattukudy 1982) and for restoring suberization following targeted inhibition of ABA biosynthesis in discs of potato (Lulai et al. 2008). The treatment solutions were decanted and the discs were placed on a perforated foam mat (Grip-It Shelf and Drawer Liner, MSM Industries, Smyrna, TN, USA) set on moist filter paper in Petri dishes (15-cm diameter) to wound heal. The dishes were covered with lids containing two, 1-cm diameter holes to ensure air circulation. The Petri dishes were placed in the dark (23°C) for 6 days and the filter papers re-moistened as needed during the wound-healing period to maintain humidity at ca. 99%. Treatments (6- and 18-month-old

tubers, two concentrations of ABA, and 6 days of healing) were arranged factorially in a randomized complete block design with three replications (3 tubers per replicate). Tuber discs were sampled daily (18 discs per sample, collectively representing 9 tubers) to assess suberization, ABA levels, protein, PAL expression and activity. Quantification of endogenous ABA concentrations was limited to non-ABA treated discs.

Determination of ABA

Changes in the ABA content of discs of tuber tissue from the 6- and 18-month-old tubers (see above) were determined following an approach similar to that of Lulai et al. (2008). Five discs were sampled daily during the 6-day healing period for each of three replicates. The discs were ground to a fine powder in liquid nitrogen using a Retsch model MM 301 ball mill (Retsch Inc., Newtown, PA, USA). A 2-g aliquot of the frozen powder was homogenized in absolute methanol (4°C), sonicated for 30 min in an ice water bath, clarified by centrifugation (10,000*g*, 15 min), and d_6-[^{2}H]-ABA was added as an internal standard. The pellet was re-extracted as above and the supernatants combined. The extract was then reduced to ca. 5 mL under nitrogen, diluted to a final methanol concentration of 70% (v/v), adjusted to pH 8.5 with NH_4OH, and applied to a 5-g C_{18} SPE column (Waters Corp., Milford, MA, USA) that had been pre-washed and pre-equilibrated with 100 and 70% methanol, respectively. The sample was eluted from the column with 75% methanol and the flow through and 75% (v/v) methanol eluates were combined. The combined eluate was diluted to $\leq$20% methanol, adjusted to pH 3.0 with 10% formic acid and applied to an Oasis HLB SPE column (Waters Corp., Millford, MA, USA) pre-washed with ether, methanol and water. Flow through from the loaded cartridge was discarded, the column was washed with 15% ethanol, and the sample was eluted with ether. The ether phase of the eluate was removed and the residual aqueous phase was re-extracted three times with additional ether. The ether phases were combined, dried under nitrogen and re-dissolved in 100 µL methanol. ABA was quantified by LC-MS-SIM by the methods of Destefano-Betran et al. (2006) (ABA *m/z* 263, d_6-[^{2}H]-ABA *m/z* 269).

Effect of ABA on wound-induced superoxide production and healing ability

The effects of exogenous ABA on wound-induced superoxide production [NADPH oxidase (NOX) activity] from 18-month-old tubers were examined using the methods of Miura et al. (1995). Tuber discs, treated with or without ABA, were placed in Petri dishes to wound heal as described above. After 16 h of healing, the discs were coated with 25 µL of Tris-buffer (0.01 M, pH 7.5) containing 500 µg mL^{-1} luminol and exposed to X-ray film to record the chemiluminescence as a measure of in vivo superoxide production (see Kumar et al. 2007b). Four replicates of discs (3 discs per sample) randomly chosen from nine tubers were examined.

The effects of tuber age and ABA treatment on the ability of parenchyma discs to develop resistance to desiccation after wounding were also determined. ABA-treated discs from 6- and 18-month-old tubers were wound healed for 6 days as described above. The healed discs were then subjected to desiccation in a forced air incubator (VWR Scientific Products, model 1575, West Chester, PA) at 45°C for 0, 10, 20, 40 and 80 min to evaluate their resistance to weight loss (Kumar and Knowles 2003). Weight loss was linear over the 80-min desiccation period. The rates of fresh weight loss provided an indirect measure of the extent of wound healing as affected by tuber age and ABA. Samples of the healed discs were also evaluated for the extent of SPP and SPA accumulation over the healing period (see below).

Suberization ratings and microscopy

The time course of suberization of parenchyma discs from the 6- and 18-month-old tubers was determined over the 6-day healing period by the methods of Lulai et al. (2008). Discs were sampled daily and placed in Farmer's fixative composed of absolute ethanol/acetic acid (3:1 v/v). Samples for each time point consisted of three replicates with each replicate made up of three discs. Analyses were done on three sections per disc with two to three ratings per section. The accumulation of suberin biopolymers was determined microscopically from 20-µm thick sections cut from the discs with a Vibratome 1000 Plus Tissue Sectioning System (The Vibratome Company, St. Louis, MO, USA). Separate suberization ratings were determined for accumulation of SPP (autofluorescence) and SPA (toluidine blue O/neutral red, Sigma Chemical Co. St. Louis, MO, USA) constituents on suberizing cell walls using methods described by Lulai and Morgan (1992) and Lulai and Corsini (1998) as modified by Lulai et al. (2006). Microscopy was performed with a Zeiss Axioskop 50 microscope configured for epifluorescent illumination as previously described (Lulai and Corsini 1998). Digital images were captured with a Zeiss color AxioCam camera (Carl Zeiss Inc., Thornwood, NY, USA). Suberization ratings spanned from zero = no accumulation, to five = accumulation about the perimeter of the first cell layer, to seven = accumulation about the first and second cell layer (Lulai and Corsini 1998; Lulai et al. 2008). The average ratings ± SE are reported (n = 3).

Table 1 Forward (F) and reverse (R) primer sequences for the amplification of transcripts of potato PAL-1 (TUBST 1, tubulin control)

Gene	Primer sequence (5′ → 3′)	Product (bp)	Accession	Reference
PAL-1	F-GCGATTTTCGCTGAAGTG R-TGTTGCTCGGCACTCTGA	596	X63103	Wang et al. (2008)
TUBST-1	F-AAATGTGCAGAACAAGAACTCATCC R-CATAACAAGTTCACTTTGGCAG	420	Z33382	Taylor et al. (1994)

RT-PCR results are shown in Fig. 4

Protein extraction, PAL activity and immunodetection

Lyophilized tuber discs were ground to a fine powder with pestle and mortar. Protein was extracted by vortexing 200 mg of lyophilized tissue in 1.0 mL of Tris-buffer (0.1 M, pH 7.2) containing 2 mM sodium bisulfite, 1 mM DTT, 0.5% (w/v) polyvinyl polypyrrolidone and 10 µL of protease inhibitor cocktail (Sigma, St. Louis, MO USA) at 4°C. The homogenate was centrifuged at 21,000*g* for 15 min (4°C). Following protein estimation (Bradford 1976), the supernatant was stored at −80°C.

PAL activity was assayed in the supernatant by the methods of Sugimoto et al. (2000) as modified by Kumar and Knowles (2003). The reaction was initiated by adding 100 µL of enzyme extract to 900 µL of HEPES buffer (100 mM, pH 8.0) containing 2 mM L-phenylalanine. The reaction was incubated at 37°C for 40 min and stopped with TCA (10% w/v, final concentration). Samples were centrifuged at 21,000*g* for 10 min prior to determining A_{290}. Concentrations of *trans*-cinnamic acid in the samples were estimated from a standard curve. PAL activity was expressed as nmol *trans*-cinnamic acid produced min^{-1} mg $protein^{-1}$.

Soluble proteins in the tuber extract were separated by SDS-PAGE (12% gels) (Laemmli 1970), electroblotted to nitrocellulose and probed with anti-pea PAL antibody diluted 1:3,000 (Sugimoto et al. 2000; Kumar and Knowles 2003). The blot was developed with alkaline phosphatase-conjugated goat secondary antibody to rabbit and visualized by incubating in $NaHCO_3$ buffer (100 mM, pH 9.8) containing 1 mM $MgCl_2$, 5.2 µM 5-bromo-4-chloro-indolyl-phosphate and 9.2 µM nitroblue tetrazolium (Kumar and Knowles 1996).

RNA extraction and RT-PCR

RNA was extracted from lyophilized tuber discs (nine tubers collectively represented in each sample) by modifying the method of Kumar et al. (2007a). Lyophilized discs were ground with pestle and mortar and 100 mg of tissue was mixed with 1.0 mL of Tris-buffer (0.1 M, pH 9.6) containing 0.65% (w/v) sodium sulfite, 0.2 M NaCl, 1% (w/v) SDS, 2% (v/v) mercaptoethanol and 10 mM EDTA. Following centrifugation at 21,000*g* for 10 min (23°C), the supernatant was mixed with an equal volume of water-saturated phenol and centrifuged as above. The resultant upper phase was extracted twice with equal volumes of chloroform:isoamyl alcohol (24:1). After centrifuging (as above), the upper phase was mixed with 0.1 volume of sodium acetate (3 M, pH 5.2) and 2.5 volumes of absolute alcohol and held at −80°C (1 h) to precipitate RNA. The pelleted RNA (21,000 g, 15 min) was washed three times with 1.5 mL of 70% aqueous ethanol, dried free of residual alcohol at 65°C and dissolved in 50 µL of DEPC-treated water. RNA was quantified at A_{260}. The integrity of RNA was determined on 1% agarose gel.

Following DNase treatment (DNA-free kit, Ambion, Inc., Austin, TX, USA), first-strand cDNA synthesis was accomplished with 5 µg of total RNA and oligo$(dT)_{20}$ primer using the Fermentas RevertAid Firststrand cDNA synthesis kit (Fermentas Inc., Glen Burnie, MD, USA) according to the manufacturer's instructions. PCR was carried out with GoTaq® DNA polymerase (Promega Corporation, Madison, WI, USA) using gene-specific primers. The forward and reverse primer sequences for PAL-1 and tubulin are given in Table 1. For PCR, samples were initially denatured at 94°C for 2 min, followed by 30 cycles of 30 s at 94°C, 30 s at 54°C, and 30 s at 72°C. Final extension was accomplished at 72°C for 5 min. Tubulin (TUBST1) served as loading control. Gels were scanned and PAL transcript levels were normalized to TUBST1.

Data analysis and presentation

Data were subjected to analysis of variance. Sums of squares were partitioned into single degree-of-freedom contrasts for main effects (tuber age, ABA, time) and their interactions, including polynomial (linear, quadratic) trends with days after wounding where appropriate. Regression coefficients and coefficients of determination are reported along with significance levels (*P* values) for correlation coefficients. Treatments were replicated three to four times depending on the study. Standard errors are reported.

Results

Tuber age affects ABA levels

Non-wounded, 6-month-old tubers contained 102 ng g^{-1} dry weight ABA compared with 13 ng g^{-1} dry weight in

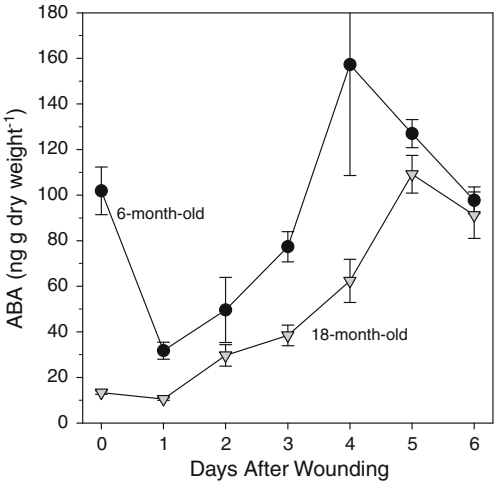

Fig. 1 ABA content in discs of parenchyma tissue from 6- (*filled circle*) and 18-month-old (*inverted triangle*) potato tubers during wound healing in the dark at 23°C (99% RH). Each *point* represents 15 discs (three replicates of five discs randomly chosen from nine tubers of each age). The effects of tuber age, days and their interaction were significant at $P < 0.01$, 0.01 and 0.05, respectively. Data are presented as ±SE

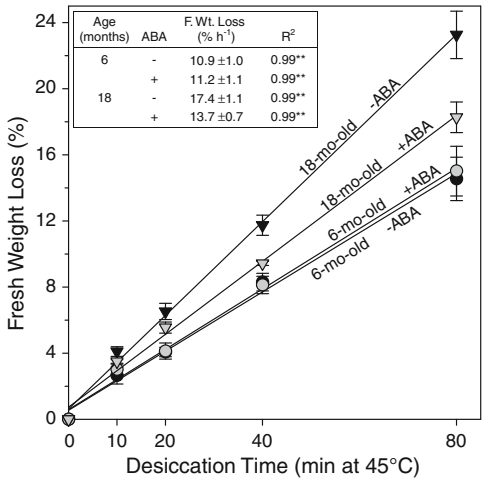

Fig. 2 Effects of tuber age and abscisic acid (*ABA*) on desiccation-induced fresh weight loss from tuber discs from 6- and 18-month-old Russet Burbank potato tubers following a 6-day period of wound healing at 23°C (99% RH). Tuber discs were immersed in 100 µM ABA or water (control) for 5 min prior to wound healing. Rates of weight loss from the healed discs are shown in the inset table (**$P < 0.01$ for correlation coefficients). The average weight loss from fresh-cut, non-healed discs (data not shown) was 35% h^{-1} ($R^2 = 0.97$, $P < 0.001$). Each point represents 18 discs (three replicates of six discs randomly chosen from nine tubers of each age). The effects of tuber age, ABA, desiccation time and their interaction were significant at $P < 0.001$. Data are presented as ±SE

18-month-old tubers, characterizing an eightfold decline in endogenous ABA concentration with tuber age (Fig. 1). ABA concentrations dropped by 69 and 22%, respectively, in 6- and 18-month-old tubers within 24 h of wounding. These initial declines were followed by linear ($P < 0.01$) increases in ABA concentrations of parenchyma discs to maximum levels at 4 days (6-month-old) and 5 days (18-month-old) after wounding, and the rate of increase depended on tuber age. ABA concentration increased by 40 ng g^{-1} dry weight day^{-1} from day 1 to 4 during healing of discs from 6-month-old tubers, as compared with 23 ng g^{-1} dry weight day^{-1} from day 1 to 5 in discs from 18-month-old tubers (age × days after wounding, $P < 0.05$). After reaching maximum levels, ABA concentrations declined in discs from both ages of tubers through day 6; however, discs from younger tubers averaged 81% higher ABA concentration than discs from older tubers over the 6-day healing period ($P < 0.01$). Tuber age thus affected the timing, rate and extent of wound-induced ABA synthesis.

Effects of tuber age and ABA on wound healing

Fresh-cut discs and discs healed for 6 days were subjected to 80 min of desiccation at 45°C and the rate of fresh weight loss compared as a relative measure of wound-healing efficiency (development of resistance to water vapor loss) (Kumar and Knowles 2003). Regardless of tuber age or ABA treatment, loss of fresh weight was linear ($P < 0.001$) over the 80-min desiccation period (Fig. 2). The rate of weight loss from fresh-cut discs from 6- and 18-month-old tubers averaged 35% h^{-1} ($R^2 = 0.97$, $P < 0.001$) (data not shown). In contrast, wound healing for 6 days substantially increased the resistance of discs to desiccation; the average rate of fresh weight loss was only 13.3% h^{-1} across treatments (Fig. 2 inset). Wound-healed discs (non-ABA treated) from 18-month-old tubers lost fresh weight 60% faster than those from 6-month-old tubers, demonstrating a significant ($P < 0.001$) reduction in this relative measure of wound-healing efficiency in older tubers (Fig. 2). ABA treatment had no effect on the development of resistance to desiccation in discs from 6-month-old tubers; however, ABA significantly enhanced the development of resistance to desiccation in discs from 18-month-old tubers (age × ABA, $P < 0.001$). While ABA-treated discs from 18-month-old tubers had 27% higher resistance to fresh weight loss than non-treated discs ($P < 0.001$), resistance remained 26% lower than in discs from 6-month-old tubers ($P < 0.01$). Thus, ABA treatment partly restored the ability to develop resistance to desiccation during wound healing of older tubers.

Tuber age, wounding and ABA affect PAL activity and expression

PAL activity was not detectable in intact tubers, but increased rapidly in response to wounding (Fig. 3). Wounding stimulated a 69% greater increase in PAL activity from

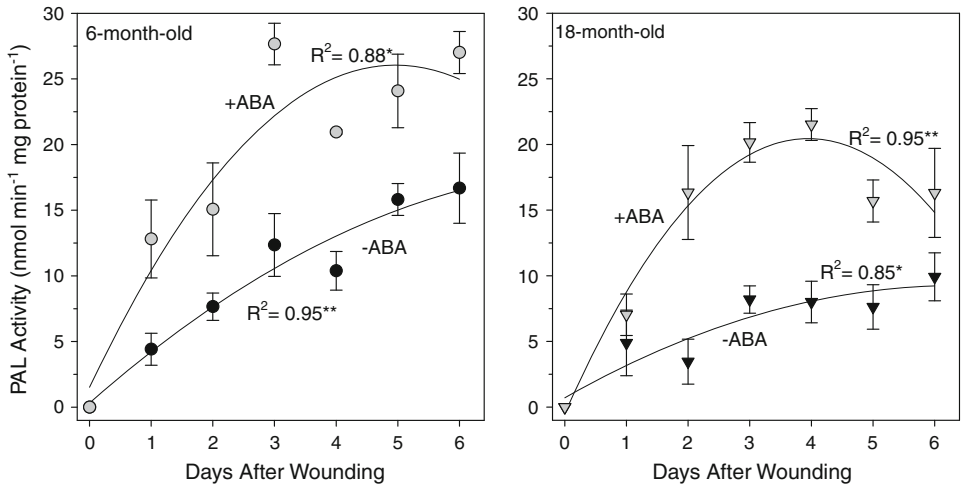

Fig. 3 Changes in phenylalanine ammonia lyase (*PAL*) activity during wound healing of 6- and 18-month-old tubers as affected by ABA. Tuber discs were immersed in 100 μM ABA or water (control) for 5 min prior to wound healing in the dark (23°C, 99% RH) for 6 days. Each *point* represents 30 discs (three replicates of ten discs randomly chosen from nine tubers of each age). Significant treatment effects included tuber age ($P < 0.01$), ABA ($P < 0.01$), days after wounding ($P < 0.01$) and the interactions of age × days ($P < 0.05$), ABA × days ($P < 0.01$), and age × days separately for non-ABA and ABA-treated discs ($P < 0.07$). *, **$P < 0.05$ and 0.01, respectively, for quadratic correlation coefficients. Data are presented as ±SE

non-ABA treated tissue from 6-month-old tubers compared to that from 18-month-old tubers over the 6-day healing interval ($P < 0.05$). ABA treatment greatly stimulated PAL activity ($P < 0.01$) regardless of tuber age. Maximum activity was reached approximately 5 and 4 days after wounding in ABA-treated discs from the 6- and 18-month-old tubers, respectively. Moreover, relative to their respective non-ABA treated control discs, ABA stimulated PAL activity to a greater extent in discs from older compared with younger tubers. Hence, tuber age did not affect the ability of discs to up-regulate PAL activity in response to exogenous ABA, and loss in the ability of older tubers to increase PAL activity in response to wounding was mostly overcome with ABA treatment.

Tuber age, wounding and ABA treatments also affected PAL-1 transcript levels (Fig. 4). In non-ABA treated discs from 6-month-old tubers, wounding induced formation of PAL-1 transcript within 24 h and expression continued to increase over the 6-day wound-healing period. Wounding also induced PAL-1 expression in non-ABA treated discs from 18-month-old tubers; however, relative to younger tubers, expression was significantly delayed and transcript levels were barely detectable until day 5 in the older tubers. ABA treatment significantly increased the expression of PAL-1 in discs from both ages of tubers over their respective controls and eliminated the 5-day delay in wound-induced expression of PAL-1 during healing of discs from older tubers. Hence, ABA treatment of wounded older tubers restored their ability to express PAL-1 to levels consistent with, or better than, non-ABA treated discs from younger tubers.

PAL protein could not be detected by Western analysis in intact tubers of either age (Fig. 4c, zero-time samples). However, PAL protein (80 kDa) accumulated in both 6- and 18-month-old tubers in response to wounding. Effects of tuber age, ABA and healing period on the amount of PAL protein could not be resolved by Western analysis.

Tuber age and ABA affect wound-induced suberization

Healthy tuber parenchyma tissue is devoid of suberized cells. Neither SPP nor SPA is present directly upon wounding (Figs. 5, 6). One day after wounding, a small amount of SPP had accumulated on the outer tangential walls of wound-responding cells from 18-month-old tubers, whereas cell walls from 6-month-old tubers accumulated discernibly more of this biopolymer (suberization ratings for SPP accumulation of 0.4 versus 1.1, respectively) (Fig. 5). This trend for slower SPP accumulation in tissues from 18-month-old tubers was clearly evident in tissue micrographs (e.g. day 4, Fig. 7a, c) throughout the wound-healing period with ratings of 4.6 versus 5.9, respectively, on the 6th day (Fig. 5). These ratings indicate that tissues from 18-month-old tubers had not yet completed SPP lamination of the first cell layer and thus significantly lagged the wound healing of 6-month-old tubers, which had laminated nearly 1.5 layers of cells with SPP by day 6.

Throughout the 6-day wound-healing time course, SPP accumulation in ABA-treated tissue from 18-month-old tubers approximately matched that from non-treated tissue derived from 6-month-old tubers (Figs. 5, 7a, d). ABA-treated tissue from 6-month-old tubers showed hastened

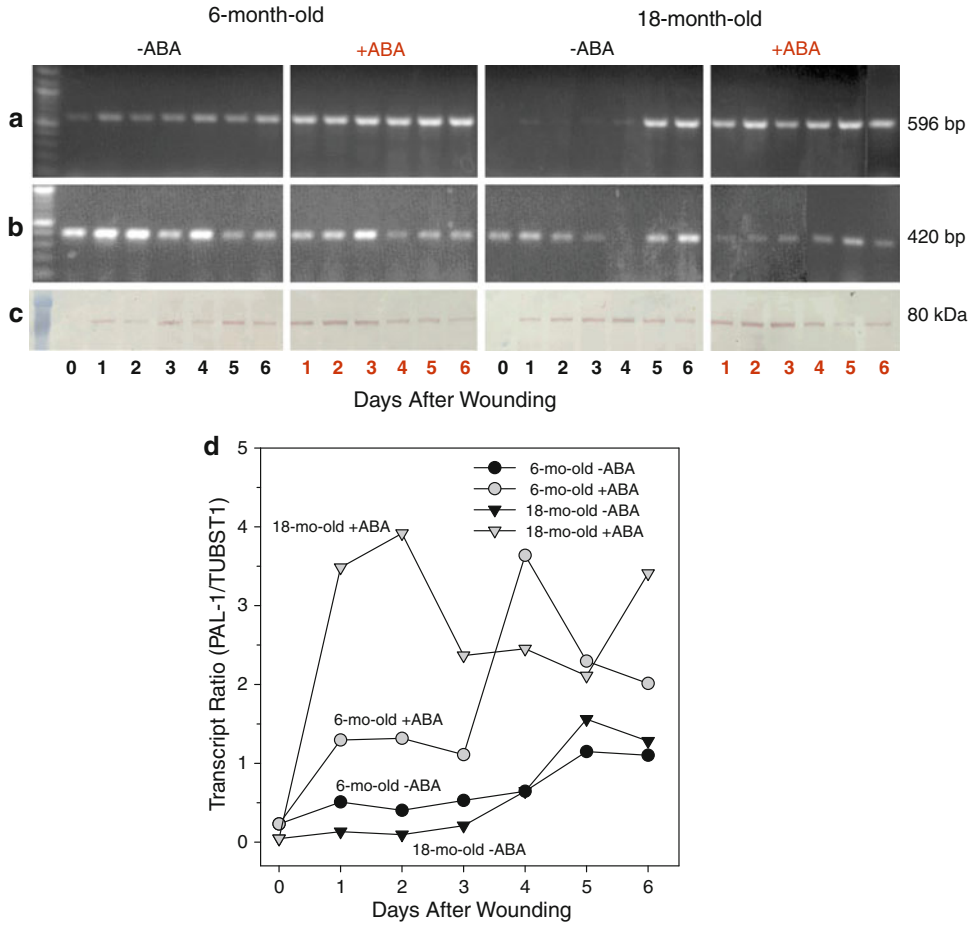

Fig. 4 Tuber age, wounding and abscisic acid (*ABA*) affect PAL-1 transcript levels (**a**) during wound healing of tissue from 6- and 18-month-old tubers. Tubulin (TUBST1) was used as a loading control (**b**). Immunodetection of PAL protein is shown in **c** (12 µg protein per lane). PAL-1 transcript levels have been normalized to TUBST1 in **d**. Tuber discs were immersed in 100 µM ABA or water (control) for 5 min prior to wound healing at 23°C (99% RH) in the *dark*. Primer sequences are presented in Table 1. Immunoblots were prepared using anti-pea PAL polyclonal antibody

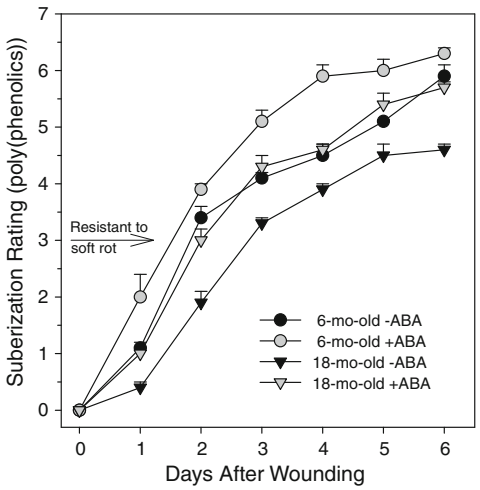

Fig. 5 Effects of tuber age and ABA on the accumulation of suberin poly(phenolics) during wound healing of 6- and 18-month-old tubers. Discs of tuber tissue were immersed in 100 µM ABA or water (control) for 5 min prior to wound healing. Each *point* represents nine discs (three replicates of three discs randomly chosen from nine tubers of each age). Tuber age, ABA, days after wounding and the interaction of age × ABA × days were significant at $P < 0.01$. Data are presented as ±SE. Micrographs of the autofluorescence of SPP on closing layer cell walls at 4 days after wounding are shown in Fig. 7

SPP accumulation over all other samples with SPP suberization ratings of 2 and 6.3 at days 1 and 6, respectively (Figs. 5, 7b). At 1.5 days after wounding, ABA-treated tissues from 6-month-old tubers reached an SPP suberization rating of three; this rating is the established point for resistance to bacterial soft rot infection where all outer tangential cell walls have accumulated SPP forming a contiguous SPP barrier. Tissues from 18-month-old tubers reached the point of resistance to soft rot at slightly less than 3 days of wound healing. Tissue from 6-month-old tubers reached the point of bacterial soft rot resistance at slightly less than 2 days of wound healing as did ABA-treated tissues from 18-month-old tubers.

Accumulation of SPA began well after SPP (Fig. 6). Discs from 18-month-old tubers did not begin to show signs of SPA accumulation until on or after the fourth day of healing. By the sixth day, initial traces of barely discernable SPA accumulations were found in the first cell layer, constituting a rating near one. ABA treatment resulted in earlier initiation of SPA accumulation, i.e. from 2 to 3 days after wounding in these older tissues. However, this ABA-induced enhancement quickly plateaued and by the sixth day of wound healing, SPA accumulation was

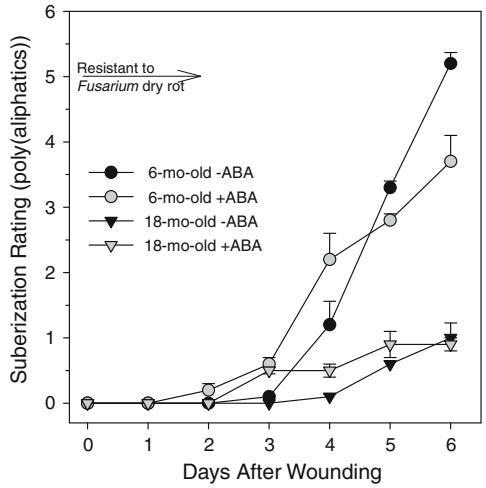

Fig. 6 Effects of tuber age and ABA on the accumulation of suberin poly(aliphatics) during wound healing of 6- and 18-month-old tubers. Discs of tuber tissue were immersed in 100 μM ABA or water (control) for 5 min prior to wound healing. Each *point* represents nine discs (three replicates of three discs randomly chosen from nine tubers of each age). Tuber age, days after wounding and the interaction of age × ABA × days were significant at $P < 0.01$. Data are presented as ±SE

equivalent to that in non-treated tissue. Regardless of ABA treatment, tissues from 18-month-old tubers failed to accumulate sufficient SPA to block fungal infections. These tissues were far short of an SPA suberization rating of five, which indicates that SPA accumulation around the first layer of cells is complete with easily discernable and bright fluorescence about the entire perimeter of the cell walls in the first cell layer.

Non-ABA treated tissues from 6-month-old tubers began to show signs of SPA accumulation after the third day of healing with initial traces of barely discernable fluorescence in the first cell layer detected by the fourth day of healing, meriting a rating of 1.2 (Fig. 6). SPA accumulation continued to rapidly increase through the fifth and to the sixth day, at which time the rating exceeded five, the point where these tissues would be resistant to fungal infection. ABA-treated tissues from 6-month-old tubers showed early signs of SPA accumulation on the second and third day of wound healing with ratings less than one. By the fourth day of wound healing, these tissues had advanced SPA accumulations with faint, but discernable SPA about the entire perimeter of the first cell layer, a rating slightly above two. The ABA-induced enhancement of SPA accumulation diminished by the fifth and sixth days of wound healing with ratings of 2.8 and 3.7, respectively; both of these ratings were lower than the non-ABA treated tissue for the same time points, i.e. 3.3 and 5.2, respectively.

Discussion

Potato tubers develop a closing layer and periderm impregnated with suberin in response to wounding, which confers substantial resistance to desiccation and microbial invasion (Vogt et al. 1983; Lulai and Orr 1994; 1995; Lulai and Corsini 1998; Matsuda et al. 2003; Schreiber et al. 2005). Suberization involves synthesis and integration of poly(phenolic) and poly(aliphatic) components into the wall matrix of the closing layer and associated phellem cells of the wound periderm. Synthesis of phenolic monomers requires induction of PAL activity (Croteau et al. 2000) and thus suberization has similarities to lignin biosynthesis, a process that is also dependent on PAL activity (Lapierre et al. 1996). Inhibition of wound-induced ABA biosynthesis in potato attenuates PAL activity and reduces accumulation of SPP, which can subsequently be restored with exogenous ABA (Lulai et al. 2008). Moreover, treatment of tuber tissue with ABA stimulates wound healing (Soliday et al. 1978). The ABA/PAL wound-signaling cascade thus partly modulates wound healing of tubers, and evidence that losses in the ability to invoke and/or respond to portions of this pathway contribute to the decline in wound-healing ability with tuber age has been provided.

Consistent with previous studies (Kumar and Knowles 2003; Kumar et al. 2007b), wound-healing ability declined with tuber age. When subjected to desiccation stress, wound-healed discs from 18-month-old tubers lost fresh weight at a significantly higher rate than those from 6-month-old tubers, characterizing a significant reduction in wound-healing ability of the older tubers (Fig. 2). Furthermore, the age-induced loss in healing ability was associated with an initial eightfold lower concentration of ABA in non-wounded older tubers (Fig. 1, zero-time), which correlated well with observed differences in the degree of sprouting of the 6- and 18-month-old tubers during storage at 4°C. While 6-month-old tubers had no visible sprouts, 18-month-old tubers had 2- to 3-mm long sprouts. Sustained ABA synthesis is required to maintain dormancy in potato tubers (Suttle and Hultstrand 1994; Biemelt et al. 2000; Destefano-Betran et al. 2006) and it is likely that the lower levels of ABA in 18-month-old tubers favored sprouting, even at temperatures non-conducive to sprout development.

The ABA content of plants in general, and potato tubers in particular, is a result of the balance between synthesis and catabolism (Kushiro et al. 2004; Destefano-Betran et al. 2006). While 9-*cis*-epoxycarotenoid dioxygenase (NCED) plays a key role in ABA synthesis in plants (Zhang et al. 2009; Xiong and Zhu 2003), catabolism is mediated by ABA-8′-hydroxylase, which converts ABA into phaseic acid (Cutler and Krochko 1999; Kushiro et al. 2004; Saito et al. 2004). The eightfold out-of-storage difference in ABA content of 6- and 18-month-old tubers (Fig. 1) is no doubt a

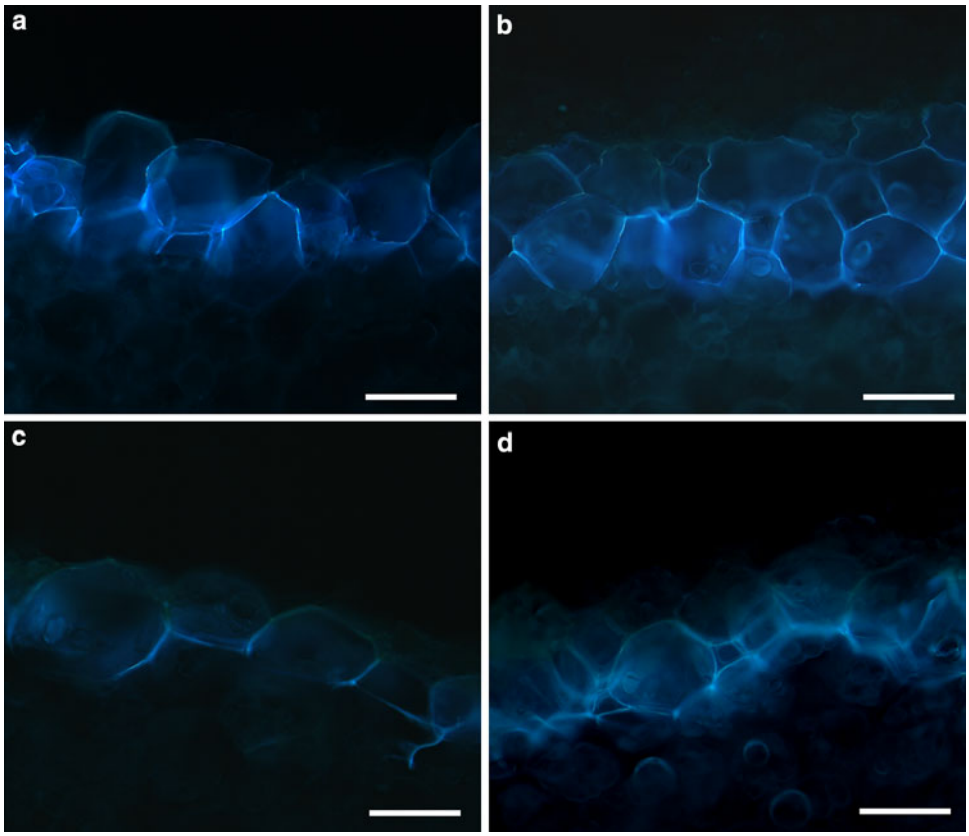

Fig. 7 Effects of tuber age and ABA treatment on suberin poly(phenolic) (*SPP*) accumulation on closing layer cell walls developing 4 days after wounding. **a** Wound-healing tissue from 6-month-old tubers; **b** ABA-treated, wound-healing tissue from 6-month-old tubers; **c** wound-healing tissue from 18-month-old tubers; and **d** ABA-treated, wound-healing tissue from 18-month-old tubers. *Scale bars* equal 50 µm. The autofluorescence is indicative of SPP accumulation in these tissues. Note the decrease in SPP accumulation on the cell walls of wound-healing tissue from 18-month-old tubers (**c**) compared to that from 6-month-old tubers (**a**). Also, note that ABA treatment of wound-healing tissue from 18-month-old tubers (**d**) increased SPP accumulation to approximate that of 6-month-old tubers (**a**). Treatment of tissue from 6-month-old tubers with ABA (**b**) further increased fluorescence (SPP accumulation) compared to non-treated tissue of the same age (**a**). The time course of SPP deposition is shown in Fig. 5

consequence of the continuous decline that begins following harvest and regulates tuber dormancy progression (Suttle 1995; Destefano-Betran et al. 2006; Lulai et al. 2008).

In agreement with the report of Lulai et al. (2008), ABA levels initially declined in response to wounding and, after 24 h, increased to maxima at 4 and 5 days of healing in discs from 6- and 18-month-old tubers, respectively (Fig. 1). However, ABA levels remained consistently lower in 18-month-old tubers over the 6-day healing period. Since endogenous ABA increased at an attenuated rate in discs from 18-month-old tubers during wound healing (Fig. 1), the ability to synthesize ABA was not totally compromised by tuber age. Increased catabolism of ABA in discs from older tubers may also have contributed to the reduced ABA accumulation during wound healing. Regardless of the mechanism, age-related differences in wound-induced ABA content appeared to affect the healing ability of older tubers through effects on PAL activity and the production of suberin biopolymers.

PAL activity is induced by a variety of factors that cause abiotic and biotic stress such as herbivory, drought, exposure to low temperature and wounding (Zucker 1968; Tanaka and Uritani 1976; Hahlbrock and Scheel 1989; Dixon and Paiva 1995; Bernards et al. 2000; Kato et al. 2000; Pociecha et al. 2009). The linear rate of wound-induced increase in PAL activity (non-ABA treated discs) was nearly twofold higher in younger compared with older tubers (2.70 vs. 1.42 nmol min^{-1} mg^{-1} protein per day) over the 6-day healing interval (Fig. 3). Exogenous ABA enhanced wound-induced PAL activity in discs from both ages of tubers, completely eliminating the age-induced difference in activity over the initial 60 h of healing. PAL activity in ABA-treated discs from older tubers was greater than in non-ABA treated (control) discs from younger tubers. Therefore, tuber age did not compromise the ability of tissue to up-regulate PAL in response to ABA. Further evidence for this is provided by the analysis of PAL-1 transcript. While wounding enhanced PAL-1 expression in both

ages of tubers, the response was significantly delayed in older tubers (Fig. 4a, d). ABA treatment completely eliminated the 5-day delay in wound-induced PAL-1 expression in discs from 18-month-old tubers, which is consistent with the notable stimulation in overall activity (Fig. 3).

PAL-1 transcript levels did not correlate as well with PAL activity during healing of older tubers compared with younger tubers. For example, the PAL-1 transcript level in non-ABA treated discs from 18-month-old tubers at day 6 was about equal to that of non-ABA treated discs from younger tubers (Fig. 4a, d), yet PAL activity was 43% lower in discs from the older compared with the younger tubers (Fig. 3). Moreover, from day 4 to 6, average PAL activity was 47% higher in ABA-treated tissue from younger tubers compared with older tubers, despite equivalent levels of PAL-1 transcript (2.6 normalized to TUBST1) averaged over this period (Fig. 4a, d). These discrepancies may be a consequence of increased protein breakdown in the older tubers. Protein content declines with tuber age, concomitant with increases in the activity of Cys-proteases (Kumar et al. 1999; Weeda 2010). The soluble protein content of 18-month-old tubers was ca. 9% lower ($P < 0.05$) than that of 6-month-old tubers. Increased catabolism of newly synthesized PAL in discs from 18-month-old tubers may have limited the ABA-induced increase in activity relative to that observed from the younger tubers.

Wound-induced de novo synthesis of PAL (Zucker 1968; Bernards et al. 2000; Kumar and Knowles 2003; Lulai et al. 2008) contributes to the development of the suberin polyphenolic (SPP) domain (Bernards 2002; Lulai et al. 2008). Consistent with the reduced wound-induced expression (Fig. 4) and activity of PAL (Fig. 3), discs from 18-month-old tubers accumulated SPP at a much slower rate than discs from 6-month-old tubers (Figs. 5, 7a, c). This difference delays SPP accumulation in the 18-month-old tubers by approximately 0.5–1.5 days behind the 6-month-old tubers during the initial 5 days of healing (Fig. 5). Thus, 18-month-old tubers took longer than 6-month-old tubers to reach the point where sufficient SPP accumulated to form a barrier to bacterial soft rot infection. ABA treatment completely abolished the age-related difference in the ability to accumulate SPP; a response consistent with the ABA-mediated up-regulation of PAL expression in the 18-month-old tubers (Figs. 3, 4, 7a, d).

ABA treatment of discs from 6-month-old tubers also enhanced the production of SPP over non-treated discs without affecting buildup in resistance to desiccation (Fig. 2), indicating that SPP was not limiting to wound healing in the younger tubers. This response may reflect the fact that the 'younger' tubers were already 6 months old and thus had lost some ability to produce and respond to endogenous ABA with SPP production in response to wounding. Involvement of ABA in the induction and accumulation of SPP has been reported previously (Lulai et al. 2008). Collectively, these results indicate that the reduction in SPP accumulation with tuber age is primarily due to reduced ability to produce ABA in response to wounding, rather than to loss of ability to respond to ABA for downstream events leading to the development of the SPP domain of suberin.

While SPP deposition guards the wounded tissue against bacterial infection (Lulai and Corsini 1998; Lulai et al. 2008), the SPA domain is responsible for containing fungal infections (Lulai 2005; Lulai et al. 2006; Thomas et al. 2007; Lulai et al. 2008). Similar to SPP accumulation, SPA accumulation during wound healing was slower in discs from older tubers compared with younger tubers (Fig. 6). However, induction of SPA accumulation in the discs from 18-month-old tubers was more severely limited than for SPP, particularly from day 4 onward. After the fourth day, the ABA-mediated enhancement of SPA accumulation in 6-month-old tuber tissue was lost; this may have been due to catabolism of the exogenous ABA (Cutler and Krochko 1999; Kushiro et al. 2004; Saito et al. 2004) or to an age-related decoupling of the regulation of SPA by ABA, which may involve deterioration of ABA receptors and binding specific for SPA.

Although ABA treatment induced earlier SPA accumulation in 18-month-old tuber tissue, this accumulation did not rapidly advance on the wound-healing cell walls as in the younger tissue. Also, unlike ABA-enhanced SPP accumulation in 18-month-old tuber tissue, ABA treatment did not enhance SPA accumulation in the older tuber tissues to the point where it matched the non-treated 6-month-old tubers. Therefore, ABA failed to completely restore the wound-healing ability of 18-month-old tubers to equal that of 6-month-old tubers (Fig. 2). The above indicated disparity for ABA-induced enhancement of SPP versus SPA accumulation in 6- and 18-month-old tuber tissues may result from different chemistries in older tissues, including the need for more ABA by the fifth and sixth day of wound healing and/or deficiency of another regulatory factor for SPA that is not as tightly linked to ABA-induced SPP accumulation. The wound-healing tissues from 18-month-old tubers did not accumulate sufficient SPAs to block fungal infection (i.e. a rating of five, Fig. 5), which indicated that SPA accumulation was complete about the first cell layer and was characterized by easily discernable and bright/intense fluorescence about the entire perimeter of the first layer of suberizing cells. Increased susceptibility to bacterial and fungal infections has been demonstrated during wound healing of 18-month-old tubers (see Fig. 8) (Kumar and Knowles 2003; Kumar et al. 2007a, b).

ABA treatment also failed to restore the ability of older tubers to induce NOX and produce superoxide at the wound site to the same extent as that observed in younger tubers

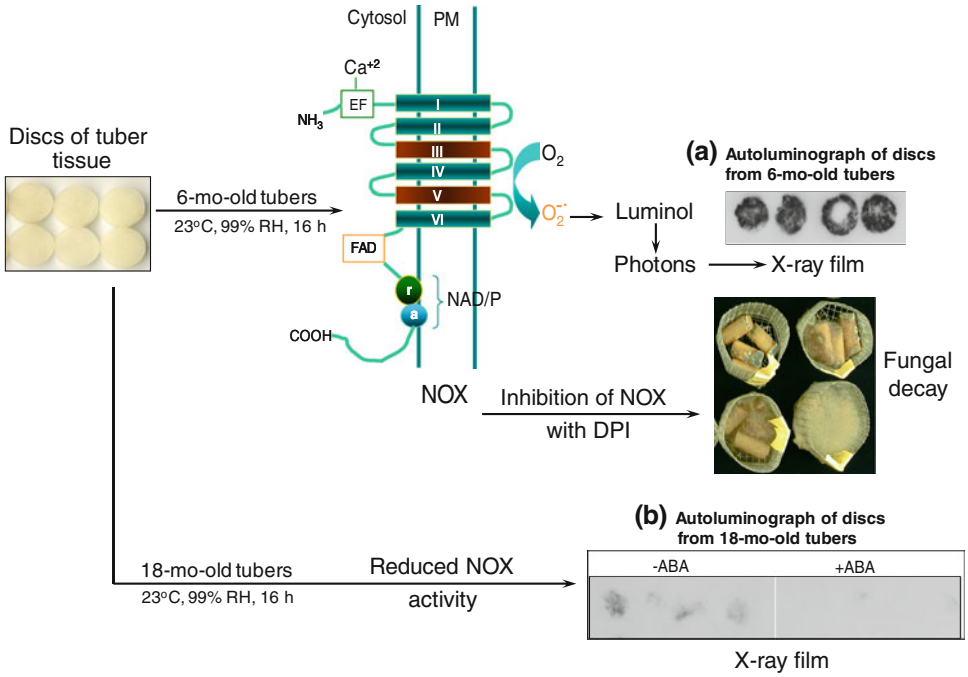

Fig. 8 Schematic illustrating the effects of tuber age on wound-induced superoxide production from discs of tuber tissue. Discs cut from 6- and 18-month-old tubers were wound healed for 16 h (23°C, 99% RH) and then treated with luminol (see "Methods"). Wounding induces NADPH oxidase (*NOX*), which generates superoxide radicals at the wound site. NOX activity increases to a maximum at approximately 24 h after wounding then decreases to pre-wounding levels by 70 h (Kumar et al. 2007b). The luminol treated discs were placed on X-ray film in the dark. The wound-induced superoxide reacts with luminol to emit photons and expose X-ray film, resulting in tissue prints (autoluminographs) of superoxide production from the discs. Autoluminographs of healing discs from 6- and 18-month-old tubers are shown in **a** and **b**, respectively. Previous work has shown that inhibition of superoxide formation by diphenyleneiodonium chloride (*DPI*) enhances susceptibility of the tuber tissue to fungal infection (note decay of cores of tuber tissue treated with DPI) (Kumar et al. 2007b). Wound-induced superoxide production is greatly reduced in 18-month-old tubers (**b**) and cannot be restored with ABA treatment, even though ABA enhanced the accumulation of SPP (Fig. 5) and wound-healing ability (see Fig. 2). Hence, the extra transient burst of wound-induced superoxide radicals characteristic of younger tubers is not necessary for the oxidative coupling of phenolics into the poly(phenolic) domain of suberin during healing. Wound-induced NOX activities were ca. 16 and 10 nmol NADPH oxidized min^{-1} g fresh $weight^{-1}$ in discs from 6- and 18-month-old tubers, respectively (Kumar et al. 2007b). *PM* plasma membrane

(Fig. 8). The wound-induced oxidative burst from NOX increases to a maximum at approximately 24 h after wounding and then declines to pre-wounding levels by 70 h (Kumar et al. 2007b). This initial wound response is greatly attenuated with age; 18-month-old tubers have substantially reduced ability to produce superoxide in response to wounding (compare autoluminographs in Fig. 8a, b; see also Kumar et al. 2007b). Since wound-induced SPP accumulation in older tubers was restored with ABA treatment (Fig. 5) without restoring wound-induced superoxide production (Fig. 8b), the age-dependent reduction in wound-induced NOX activity was likely inconsequential with regard to the ability of older tubers to oxidatively couple phenolics into the aromatic domain of suberin. However, the reduced oxidative burst of older tubers in response to wounding greatly increases their susceptibility to decay (Fig. 8; Kumar et al. 2007b).

In summary, older tubers are less efficient at accumulating ABA in response to wounding than younger tubers, which affects the downstream metabolism critical to suberization and the development of resistance to desiccation during wound healing. The reduced wound-healing ability of older tubers can be partly rectified by treatment with ABA. Exogenous ABA corrected the transcriptional delay of PAL-1 during wound healing of older tubers, thus restoring the synthesis and accumulation of SPP. ABA did not restore the ability of older tubers to accumulate SPA, which accounts for only partial recovery in wound-healing ability of the older tubers. Further work should focus on elucidating the mechanisms of age-induced dysfunctions in wound-induced ABA metabolism and on characterizing the downstream signaling events that couple SPA biosynthesis and/or deposition to ABA. This decoupling likely explains why the age-induced loss of healing ability was only partly recoverable with ABA treatment. The aged tuber model system has allowed dissection of the role of ABA in the induction of PAL and in the regulation of SPP and SPA deposition during suberization and has thus increased our understanding of the wound-healing process in potato tubers.

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References

Bernards MA (2002) Demystifying suberin. Can J Bot 80:227–240

Bernards MA, Fleming WD, Lewellyn DB, Priefer R, Yang X, Sabatino A, Plourde GL (1999) Biochemical characterization of the suberization-associated anionic peroxidase of potato. Plant Physiol 121:135–146

Bernards MA, Susag LM, Bedgar DL, Anterola AM, Lewis NG (2000) Induced phenylpropanoid metabolism during suberization and lignification: a comparative analysis. J Plant Physiol 157:601–607

Biemelt S, Hajirezaei M, Hentschel E, Sonnewald U (2000) Comparative analysis of abscisic acid content and starch degradation during storage of tubers harvested from different potato varieties. Potato Res 43:317–382

Borchert R (1978) Simultaneous separation of acidic and basic isoperoxidases in wounded potato tissue by acrylamide gel electrophoresis. Plant Physiol 62:794–797

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cottle W, Kollattukudy PE (1982) Abscisic acid stimulation of suberization. Plant Physiol 70:775–780

Croteau R, Kutchan TM, Lewis NG (2000) Natural products (secondary metabolites). In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, pp 1250–1318

Cutler AJ, Krochko JE (1999) Formation and breakdown of ABA. Trends Plant Sci 4:472–478

Destefano-Betran L, Knauber D, Huckle L, Suttle J (2006) Effects of postharvest storage and dormancy status on ABA content, metabolism, and expression of genes involved in ABA biosynthesis and metabolism in potato tuber tissues. Plant Mol Biol 61:687–697

Diallinas G, Kanellis AK (1994) A phenylalanine ammonia-lyase gene from melon fruit: cDNA cloning, sequence and expression in response to development and wounding. Plant Mol Biol 26:473–479

Dixon RA, Paiva NL (1995) Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097

Doke N (1983) Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissue infection with an incompatible race of *Phytophthora infestans* and the hyphal wall components. Physiol Plant Path 23:345–357

Hahlbrock K, Scheel D (1989) Physiology and molecular biology of phenyl propanoid metabolism. Ann Rev Plant Physiol Mol Biol 40: 347–369

Hyodo H (1976) Wound-induced phenylalanine ammonia-lyase in potato tuber tissue development of enzyme activity and effects of antibiotics. J Biochem 79:277–282

Jiang YM, Joyce DC (2003) ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. Plant Growth Regul 39:171–174

Johnson SM, Doherty SJ, Croy RRD (2003) Biphasic superoxide generation in potato tubers. A self-amplifying response to stress. Plant Physiol 131:1–10

Kato M, Hayakawa Y, Hyodo H, Ikoma Y, Yano M (2000) Wound-induced ethylene synthesis and expression and formation of 1-amiocyclopropane-1-carboxylate (ACC) synthase, ACC oxidase, phenylalanine ammonia-lyase, and peroxidase in wounded mesocarp tissue of *Cucurbita maxima*. Plant Cell Physiol 41:440–447

Kumar GNM, Knowles NR (1996) Oxidative stress results in increased sinks for metabolic energy during aging and sprouting of potato seed-tubers. Plant Physiol 112:1301–1313

Kumar GNM, Knowles NR (2003) Wound-induced superoxide production and PAL activity decline with potato seed-tuber age and wound-healing ability. Physiol Plant 117:108–117

Kumar GNM, Houtz RL, Knowles NR (1999) Age-induced protein modifications and increased proteolysis in potato seed-tubers. Plant Physiol 119:89–99

Kumar GNM, Iyer S, Knowles NR (2007a) Extraction of RNA from frozen and lyophilized tuber and root tissues. J Agric Food Chem 55:1674–1678

Kumar GNM, Iyer S, Knowles NR (2007b) Strboh a homologue of NADPH oxidase regulates wound-induced oxidative burst and facilitates wound-healing in potato tubers. Planta 227:25–36

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E (2004) The Arabidopsis cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. EMBO J 23:1647–1656

Laemmli UK (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227:680–685

Lapierre C, Pollet B, Negrel J (1996) The phenolic domain of potato suberin: structural comparison with lignins. Phytochem 42:949–953

Lewis NG, Yamamoto E (1990) Lignin: occurrence, biogenesis and biodegradation. Annu Rev Plant Physiol Plant Mol Biol 41:455–496

Lulai EC (2005) Non-wound induced suberization of tuber parenchyma cells: a physiological response to the wilt disease pathogen *Verticillium dahliae*. Am J Potato Res 82:433–440

Lulai EC (2007) The canon of potato science: skin-set and wound-healing/suberization. Potato Res 50:387–390

Lulai EC, Corsini DL (1998) Differential deposition of suberin phenolic and aliphatic domains and their roles in resistance to infection during potato tuber (*Solanum tuberosum L.*) wound-healing. Physiol Mol Plant Path 53:209–222

Lulai EC, Morgan WC (1992) Histochemical probing of potato periderm with neutral red: a sensitive cytofluorochrome for the hydrophobic domain of suberin. Biotech Histochem 67:185–195

Lulai EC, Orr PH (1994) Techniques for detecting and measuring developmental and maturational changes in tuber native periderm. Am Potato J 71:489–505

Lulai EC, Orr PH (1995) Porometric measurements indicate wound severity and tuber maturity affects the early stages of wound-healing. Am Potato J 72:225–241

Lulai EC, Weiland JJ, Suttle JC, Sabba RP, Bussan AJ (2006) Pink eye is an unusual periderm disorder characterized by aberrant suberization: a cytological analysis. Am J Potato Res 83:409–421

Lulai EC, Suttle JC, Pederson SM (2008) Regulatory involvement of abscisic acid in potato tuber wound-healing. J Exp Bot 58:1175–1186

Matsuda F, Morino K, Miyashita M, Miyagawa H (2003) Metabolic flux analysis of the phenylpropanoid pathway in wound-healing potato tuber tissue using stable isotope-labeled tracer and LC-MS spectroscopy. Plant Cell Physiol 44:510–517

Miura Y, Yoshioka H, Doke N (1995) An autophotographic determination of the active oxygen generation in potato tuber discs during hypersensitive response to fungal infection or elicitor. Plant Sci 105:45–52

Pociecha E, Płażek A, Janowiak F, Waligórski P, Zwierzykowski Z (2009) Changes in abscisic acid, salicylic acid and phenylpropa-

noid concentrations during cold acclimation of androgenic forms of *Festulolium* (*Festuca pratensis X Lolium multiflorum*) in relation to resistance to pink snow mould (*Microdochium nivale*). Plant Breed 128:397–403

Razem FA, Bernards MA (2003) Reactive oxygen species production with suberization: evidence for an NADPH-dependent oxidase. J Exp Bot 54:935–941

Saito S, Hirai N, Matsumoto C, Ohigashi H, Ohta D, Sakata K, Mizutani M (2004) Arabidopsis CYP707As encode (+)-abscisic acid 8′-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. Plant Physiol 134:1439–1449

Schreiber L, Franke R, Harmann K (2005) Wax and suberin development of native and wound periderm of potato (*Solanum tuberosum* L.) and its relation to peridermal transpirations. Planta 220:520–530

Soliday CL, Dean BB, Kolattukudy PE (1978) Suberization: inhibition by washing and stimulation by abscisic acid in potato disks and tissue culture. Plant Physiol 61:170–174

Sugimoto M, Toyoda K, Ichinose Y, Yamada T, Shiraishi T (2000) Cytochalasin A inhibits phenylalanine ammonia lyase mRNA to ribosomes during induction of phytolalexin in pea seedlings. Plant Cell Physiol 41:234–238

Suttle JC (1995) Postharvest changes in endogenous ABA levels and ABA metabolism in relation to dormancy in potato tubers. Physiol Plant 95:233–240

Suttle JC, Hultstrand JF (1994) Role of endogenous abscisic acid in potato microtuber dormancy. Plant Physiol 105:891–896

Tanaka Y, Uritani I (1976) Immunochemical studies on fluctuation of phenylalanine ammonia-lyase activity in sweet potato in response to cut injury. J Biochem 79:21

Taylor MA, Wright F, Davies HV (1994) Characterisation of the cDNA clones of two beta-tubulin genes and their expression in the potato plant (*Solanum tuberosum* L.). J Plant Mol Biol 26:1013–1018

Thomas R, Fang X, Ranathunge K, Anderson TR, Peterson CA, Bernards MA (2007) Soybean root suberin: anatomical distribution, chemical composition, and relationship to partial resistance to *Phytophthora sojae*. Plant Physiol 144:299–311

Vogt E, Schonher J, Schmidt HW (1983) Water permeability of periderm membranes isolated enzymatically from potato tubers (*Solanum tuberosum* L.). Planta 158:294–301

Wang X, El Hadrami A, Adam LR, Daayf F (2008) Differential activation and suppression of potato defense responses by Phytophthora infestans isolates representing US-1 and US-8 genotypes. Plant Pathol 57:1026–1037

Weeda SM (2010) Protein turnover in potato tubers during development, long-term storage, and plant establishment. PhD dissertation, Washington State University, Mt Vernon

Xiong L, Zhu JK (2003) Regulation of abscisic acid biosynthesis. Plant Physiol 133:29–36

Zhang M, Yuan B, Leng P (2009) Cloning of 9-*cis*-epoxycarotenoid dioxygenase (NCED) gene and the role of ABA on fruit ripening. Plant Signal Behav 4:460–463

Zucker M (1968) Sequential induction of phenylalanine ammonia-lyase and a lyase-inactivating system in potato tuber disks. Plant Physiol 43:365–374